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Folate metabolism in malaria

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It is known that malaria parasites are inhibited by sulfonamides and antifolate compounds, require 4-aminobenzoic acid for growth, and respond only partly to intact folic and folinic acids. Biochemical data obtained during the last decade on the synthesis of nucleic acid precursors and on folate enzymes in malaria support the hypothesis that malaria parasites are similar to microorganisms that synthesize folate cofactors de novo. *Sulfa drugs inhibit plasmodial dihydropteroate synthase (EC 2.5.1.15). Pyrimethamine and many other antifolate compounds bind to tetrahydrofolate dehydrogenase (EC 1.5.1.3) of the parasite more tightly than to the host enzyme. However, the metabolic consequences of the depletion of folate cofactors as a result of drug inhibition are not yet known. Other areas to be studied are the origin of the pteridine moiety of folates, the addition of glutamate(s) in folate cofactor biosynthesis, the means by which intact, exogenous folates affect malarial growth, and demonstration of the enzymes and reactions involving* N^5-*methyl tetrahydrofolate.*

Studies on the sulfonamides and 4-aminobenzoic acid (pABA) in malaria date back about four decades, when sulfachrysoidine and sulfanilamide were first used to treat human malarial infections (*1*) and pABA was found to reverse sulfonamide inhibition in *Plasmodium gallinaceum* (*2*). Since then, there have been many reports of studies on the effects of folate precursors, folate metabolites, folate end-products, and folate analogues on malarial growth and survival *in vivo* and *in vitro*. Most of these data are consistent with the current general view of folate metabolism in most bacteria; these organisms synthesize the needed folate cofactors *de novo* and do not utilize the exogenously supplied, intact folate molecule (*3*). The enzymatic evidence accumulated in the last decade supports this view, so that the general outline of the origin of folate cofactors within the malarial parasite seems to be established. However, there are still questions to be answered on the details of the enzymes involved, on the functions of folate cofactors in malaria, on the mechanism by which exogenous folates exert their effects in malaria, and on the physiological consequences of the inhibition of folate cofactor biosynthesis by sulfonamides and antifolate drugs. This article reviews what is known and what questions remain unanswered on folate metabolism in malaria.

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DIHYDROFOLATE BIOSYNTHESIS [a]

The growth of plasmodia is greatly stimulated by pABA, *in vivo* and *in vitro*. The growth of *P. knowlesi* in the Harvard medium developed during World War II (*4*) required pABA, which prevented inhibition of growth by sulfadiazine (*5*). Erythrocytic forms of several species of plasmodium grew poorly or not at all in hosts fed on pABA-deficient diets. Hawking (*6*) reported the suppression of parasitaemia of *P. berghei* in the rat and *P. cynomolgi* in the rhesus monkey fed on milk diets, which were shown to have a low pABA content. In both cases, pABA supplementation of the milk diet allowed the infections to develop. Other reports followed, with varying results, including some sulfonamide-resistant strains of *P. berghei* that grew well in mice on a milk diet —see Peters (*7*) for a review. Some of the variability observed could be due to differences between strains in their capacity to utilize low levels of pABA, since Vray (*8*) found that, in mice on a pABA-deficient diet, blood levels of pABA fell rapidly but did not completely disappear. The pABA level in the liver remained high, allowing normal development of hepatic schizonts after sporozoite inoculation.

[a] Abbreviations used: pABA (4-aminobenzoic acid); pABG (4-aminobenzoylglutamate); H_2-folate (7,8-dihydropteroylglutamate); H_2pteroate (7,8-dihydropteroate); pt (2-amino-4-hydroxy-pteridine); H_2ptCH_2OH (6-hydroxymethyl-7,8-dihydro-pt); H_2ptCH_2OPP (6-pyrophosphorylmethyl-7,8-dihydro-pt); H_4-folate (5,6,7,8-tetrahydropteroylglutamate); folinic acid (N^5-formyl-H_4-folate).

The pABA requirement of *P. falciparum* has been indicated by studies of Kretschmar, who suggested that the low incidence of *P. falciparum* in children fed on milk diets was due to the low content of pABA in milk (*9*). Kretschmar & Voller (*10*) later showed that *P. falciparum* did not grow in *Aotus* monkeys maintained on a milk diet. The experiment would have been more conclusive, however, had it demonstrated that the parasite would grow if pABA were included in the milk diet.

Jacobs (*11*) published a detailed study and discussion of the role of pABA in malarial infections, *in vivo*. He observed increasing parasitaemia in mice infected with *P. berghei* (NYU-2 strain) with increasing pABA levels in the diet, and no growth in its absence. A pyrimethamine-resistant strain required higher levels of pABA for optimum development. Folic and folinic acids only partially replaced pABA. Jacobs concluded that "... the parasite is unable to utilize the preformed folic or folinic acid molecule and that pABA is used by the parasite to form a substance similar to, but not identical with, folic acid." (*11*). On the basis of our current knowledge on folate biosynthesis, that substance would appear to be dihydrofolate.

The competitive nature of the reversal by pABA of sulfonamide inhibition has been documented in several species of plasmodia (*7*, *12*). By analogy with the metabolic pathways established in bacteria, this should mean that the sulfa drugs compete with pABA for binding to the enzyme dihydropteroate synthase (EC 2.5.1.15) (*3*). This enzyme catalyses the condensation of pABA with H_2ptCH$_2$OPP to form H_2 pteroate (Fig. 1) and is the site of action of the sulfonamides in bacteria. The enzyme has been found in extracts of *P. chabaudi* (*14*), *P. berghei* (*15*, *16*), and probably *P. knowlesi*, *P. lophurae*, and *P. gallinaceum* (*15*). The three last-mentioned species were assayed with pABG in place of pABA as substrate; pABG has been shown to be an alternative substrate to pABA in bacteria and plants (*13*) and *P. berghei* (*15*).

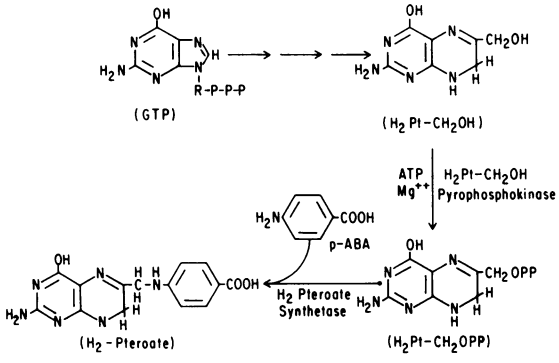

Fig. 1. Synthesis of H2pteroate by bacteria and plants (*13*), showing the reactions of 2-amino-4-hydroxy-6-hydroxymethyldihydropteridine pyrophosphokinase (EC 2.7.6.3) and dihydropteroate synthase (EC 2.5.1.15).

The enzyme before dihydropteroate synthase in the pathway of H_2folate biosynthesis is 2-amino-4-hydroxy-6-hydroxymethyldihydropteridine pyrophosphokinase (EC 2.7.6.3) (Fig. 1). In *Escherichia coli* these two enzymes are separated by DEAE-cellulose or Sephadex G-100 chromatography (*13*). In *Lactobacillus plantarum* there was evidence both for a complex and for a separate dihydropteroate synthase from a DEAE-cellulose column, (*17*), and in pea seedlings the two activities resided in a single protein (*18*). I found that these two enzymatic activities coeluted when a *P. berghei* crude extract was run on a Sephadex G-200 column, but they could be separated by DEAE-Sephadex chromatography (*15*). Walter & Königk reported that they could not separate the synthase and pyrophosphokinase activities from *P. chabaudi* during a purification scheme that resulted in a 950-fold increase in specific activity (*19*). However, they did not use an assay that would have detected the pyrophosphokinase activity alone, in the crude extracts or early fractions. The combined assay used determines the specific activity of the limiting reaction only, and, if a separate pyrophosphokinase were present in excess of the dihydropteroate synthase specific activity, it would not have been detected. The protein fraction they isolated carried out the combined reaction, and the two activities were found together on Sephadex column chromatography of a *P. berghei* crude extract, so it is likely that complexes of these enzymatic activities exist in rodent plasmodia. Several other differences were found for these enzymes in the studies reported (Table 1), which may be due to species differences, the presence or absence of the enzyme complex in the fractions studied, or differences in methodology. Most importantly though, two key enzymatic reactions required for dihydrofolate biosynthesis have been demonstrated in malarial parasites.

The inhibition of malarial dihydropteroate synthase by sulfonamides and dapsone and their competition with pABA have also been reported (Table 1). The inhibition constants (K_i values) obtained are comparable to these determined with the enzyme from bacterial sources (*13*) and satisfactorily con-

Table 1. Properties of malarial dihydropteroate synthases

Source	Substrate	Reference	Sp. act. (pmol/min/mg protein)	pH optimum	Apparent K_m values (μmol/litre)				Apparent K_i values (μmol/litre)			
					H_2ptCH_2OH	H_2ptCH_2OPP	pABA	pABG	DDS	sulfadiazine	sulfathiazole	sulfanilamide
P. berghei	H_2ptCH_2OH [a]	(*15*)	1	7.0	0.8	—	0.28	37	0.38	1.4 [b]	0.56	25.2
	H_2ptCH_2OPP	(*21*)	1	ND [c]	—	1.4	0.21	ND	ND	ND	ND	ND
P. berghei	H_2ptCH_2OPP	(*16*)	2	8.5	—	1.4	2.8	ND	19 [b]	39 [b]	ND	ND
P. chabaudi	H_2ptCH_2OH	(*19*)	16.5	8.7	11.0	—	1.5	ND	ND	ND	1.4	13.0
	H_2ptCH_2OPP	(*19*)	16.0	8.7	—	ND	ND	ND	ND	ND	ND	ND

[a] Combined assay method for malarial 2-amino-4-hydroxy-6-hydroxymethyldihydropteridine pyrophosphokinase and dihydropteroate synthase.
[b] Calculated value.
[c] Not determined.

firm, on an enzymatic basis, the sulfa drug/pABA interrelationship observed in malaria *in vivo* and *in vitro*. Investigation of the properties of dihydropteroate synthase in sulfonamide-resistant strains of malaria might be very useful in determining the mode(s) of resistance to these compounds in malaria, but so far no such investigations have been reported.

In malaria, H_2-folate biosynthesis may be inhibited by compounds other than pABA analogues, since one compound with antimalarial activity appears to be a pteridine analogue. On the basis of its inhibition of pyrimethamine-resistant *P. cynomolgi*, Kisliuk et al. (*20*) proposed that tetrahydrohomopteroate may interfere with H_2folate biosynthesis in malaria. I found that both tetrahydro- and dihydrohomopteroate were strong inhibitors of the activity of *P. berghei* dihydropteroate synthase (*21*). The latter was competitive with H_2ptCH_2OPP, with an apparent K_i value of 7 nmol/litre.

Gaps still remain in our knowledge of the pathway of H_2folate biosynthesis in malaria. No evidence has been published on the origin of the pteridine. In bacteria and plants, H_2ptCH_2OH is synthesized from guanosine triphosphate in a series of enzymatic steps (*13*). Plasmodia might contain these enzymes, or perhaps the H_2ptCH_2OH is obtained as a result of the cleavage of folates by the host or parasite. This pteridine was produced by Chinese hamster ovary cells in a culture medium containing folic acid (*22*). Plasmodia presumably do not synthesize pABA, since nutritional studies indicate that it must be supplied in the diet.

Dihydrofolate synthetase (EC 6.3.2.12), which catalyses the reaction H_2pteroate+L-glutamate+ATP $\xrightarrow{Mg^{++}}$ H_2folate+ADP+orthophosphate has not yet been reported in malaria. We have failed to demonstrate its presence in crude or partially purified extracts of *P. berghei* (R. Ferone & S. Roland, unpublished observations, 1976) by either microbiological or radiochemical assay methods (*23*). Dihydropteroate synthase from *P. berghei* uses pABG as an alternative substrate to form H_2folate directly, but the apparent K_m for pABG is about 100 times higher than that for pABA. The pABG might arise from the cleavage of folates, but this would not seem to be a normal process in plasmodia because of the readily observed dietary requirement of pABA. However, it is possible that the numerous observations on the effects of high levels of folic and folinic acids in growth and inhibitor studies can be explained by cleavage of the intact molecules and utilization of the pABG (and possibly the pteridine). Rollo (*24*) proposed this as an explanation for the observation of competitive reversal by folic acid of sulfonamide inhibition of *P. berghei* in the mouse, instead of the non-competitive reversal that might have been expected if folic acid had been used intact. However, if pABG is not normally used by the parasite, then an enzyme that adds glutamate to H_2pteroate should be present in plasmodia and remains to be demonstrated, since most of the folates of *P. berghei* contain one or more glutamates (*25*).

TETRAHYDROFOLATE DEHYDROGENASE (EC 1.5.1.3)

Tetrahydrofolate dehydrogenase is a key enzyme in folate metabolism because it converts the pteridine ring to the tetrahydro reduction state required for

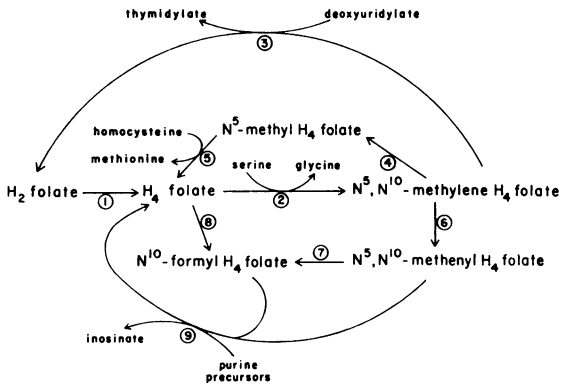

Fig. 2. Folate cofactor synthesis and utilization studied in plasmodia. (1) tetrahydrofolate dehydrogenase (EC 1.5.1.3); (2) serine hydroxymethyltransferase (EC 2.1.2.1); (3) thymidylate synthase (EC 2.1.1.45); (4) 5,10 methylenetetrahydrofolate reductase (EC 1.1.1.68); (5) tetrahydropteroylglutamate methyltransferase (EC 2.1.1.13); (6) methylenetetrahydrofolate dehydrogenase (EC 1.5.1.5); (7) methenyltetrahydrofolate cyclohydrolase (EC 3.5.4.9); (8) formyltetrahydrofolate synthetase (EC 6.3.4.3); (9) purine biosynthetic enzymes involving folate cofactors: phosphoribosylglycinamide formyltransferase (EC 2.1.2.2) and phosphoriboselaminoimidazolecarboxamide formyltransferase (2.1.2.3).

the reactions in which the folate cofactors are synthesized and utilized (Fig. 2). It was the first folate enzyme demonstrated in plasmodia (*27*), and has since been reported in many species of malaria parasite (*28–31*). Many of the properties of enzymes from the drug-sensitive strains reported are alike (Table 2), including an apparent molecular weight of approximately 100 000–200 000, determined by gel filtration. The molecular weight of the malarial tetrahydrofolate dehydrogenase is 5–10 times as high as the molecular weights found for the enzyme from most other sources (bacteria, avies, mammals (*26*), and helminths (*32*)), but is in the same range as that found for the enzyme from trypanosomes (*32*), *Eimeria tenella* (*33*), *Tetrahymena pyriformes*, *Hartmanella castellani*, *Euglena gracilis*, and *Leishmania donovani* (R. Ferone & R. Neal, unpublished observations, 1975).

Pyrimethamine binds 10^2 to 10^3 times more tightly to the plasmodial tetrahydrofolate dehydrogenase than to the enzyme from mammalian or avian sources (*27*, *29*). Similar values for tighter binding were found for trimethoprim, cycloguanil, and several dihydrotriazines with antimalarial activity (*27*, *34*). The concentrations of trimethoprim and pyrimethamine required to inhibit *P. knowlesi in vitro* are close to the concentrations inhibitory to the isolated tetrahydrofolate dehydrogenase (*28*, *35*). Thus it appears that these compounds, and probably many other antifolate compounds, act as antimalarials by selective inhibition of the plasmodial tetrahydrofolate dehydrogenase, causing a depletion of required folate cofactors.

By determining the relative inhibition values of compounds *versus* the parasite and the host enzymes, an " enzymatic chemotherapeutic index ", which is

Table 2. Properties of malarial tetrahydrofolate dehydrogenase

Plasmodium species	References	Sp. act. (nmol/min/mg protein)	Apparent K_m H_2folate (μmol/litre)	50 % inhibitory concentration pyrimethamine (nmol/litre)
berghei	*27, 31, 34, 56, 57*	0.8–3.9	1.6–4.2	0.5–1.0
berghei, pyrimethamine-resistant	*34, 56, 57*	5.0–39	9.0–51.9	20–30
vinckei	*31, 57*	4.8, 8.0	4.9, 7.9	2.0
vinckei, pyrimethamine-resistant	*31, 57*	17.4, 49	17.1, 68.0	450
chabaudi	*30*	—	5.0	—
knowlesi	*28*	1–3	3.0	1.0
lophurae,				
uninucleate	*29*	1.76	—	—
multinucleate	*29*	4.89	1.4	6.0

crudely predictive of antimalarial activity *in vivo*, may be obtained. Compounds that do not selectively inhibit the plasmodial enzyme would not be highly effective *in vivo*, because of low antimalarial potency or high host toxicity. Several analogues of trimethoprim with superior activity against *P. berghei in vivo* have been found by this means (*36*). A diaminoquinazoline that was extensively studied as an antimalarial (WR 158122) (*37*) inhibits rat liver tetrahydrofolate hydrogenase 17 times as strongly as pyrimethamine (*38*) and is half as potent against the *P. berghei* enzyme (R. Ferone, D. Cleland, unpublished observations, 1974). Thus, if the pharmacokinetic parameters of the two compounds were similar, the diaminoquinazoline would be less selective and potentially more toxic than pyrimethamine. If this were not the case, it would indicate favourable pharmacokinetic properties for the former and suggest a profitable area for study.

Tetrahydrofolate hydrogenases with altered properties have been found in several pyrimethamine-resistant strains of *P. berghei* and *P. vinckei*. In all cases, the enzyme-specific activity was increased, and the binding of H_2folate and of pyrimethamine decreased (Table 2). Thus in these highly resistant strains, the mechanism of resistance appears to be increased production of an enzyme with decreased affinity for the drug. The increased K_m for H_2folate is consistent with the increased pABA requirement of a pyrimethamine-resistant strain of *P. berghei* noted by Jacobs (*11*). Such a strain was found to be cross-resistant to trimethoprim and to cycloguanil, both *in vivo* and on an enzymatic basis (*34*). Obviously, many other mechanisms of resistance are possible (including increased substrate level, decreased folate cofactor requirement, utilization of folate end-products, decreased permeability to drug, and drug metabolism), and it is likely that some of these will be found to account for the low level of resistance frequently encountered in *P. falciparum* in man.

TETRAHYDROFOLATE COFACTOR SYNTHESIS AND UTILIZATION

Fig. 2 shows the reactions involving H_4folate cofactor synthesis and function that have been studied in malaria. Other reactions are known, but there are no reports of their investigation in plasmodia. Platzer (*39*) found that the activities of formyltetrahydrofolate synthetase (EC 6.3.4.3) and of methylenetetrahydrofolate dehydrogenase (EC 1.5.1.5) were decreased in duckling erythrocytes infected with *P. lophurae*, and neither enzymatic activity could be demonstrated in extracts of free parasites. While Platzer realized the difficulty of trying to prove the absence of enzymes in cells, he pointed out that the absence of these two reactions was consistent with the apparent inability of malarial parasites to synthesize purines *de novo*.

Elevated levels of serine hydroxymethyltransferase (EC 2.1.2.1) were found in the parasitized erythrocytes and the enzyme was demonstrated in extracts of free parasites (*39*). The parasite enzyme differed from that of the host in molecular weight, pH optimum, and stability. N^5,N^{10}-methylenetetrahydrofolate produced from serine hydroxymethyltransferase is the folate cofactor required for the methylation of deoxyuridylate by thymidylate synthase (EC 2.1.1.45) (Fig. 2). Since malaria parasites do not incorporate exogenous thymine or thymidine, the thymidylate required for DNA synthesis must be synthesized *de novo*, presumably by thymidylate synthase. This enzyme has been found in extracts of *P. lophurae* (*40*), *P. chabaudi* (*30*), and *P. berghei* (*41*). The specific activity in *P. chabaudi* was one-tenth that of tetrahydrofolate dehydrogenase; the amounts of both increased in parallel during development, particularly during the early phase of schizogony (*42*). The molecular weight of the *P. berghei* thymidylate synthase was $>100\,000$, in contrast to a value of 68 000 for the mouse reticulocyte enzyme (*41*). Reid & Friedkin pointed out the similarity of the high molecular weights of tetrahydrofolate dehydrogenase and thymidylate synthase in *P. berghei* (*41*).

Smith et al. (*43*) recently demonstrated the incorporation of radioactivity from [3-^{14}C]serine into thymidylate and methionine in *P. knowlesi*-infected erythrocytes, *in vitro*. Since these incorporations presumably occurred *via* serine hydroxymethyltransferase, this procedure will be useful for investigating the effects of folate analogues and precursors in growing plasmodia.

Two investigations have been published indicating the synthesis of N^5-methyl tetrahydrofolate and its utilization for methionine synthesis (Fig. 2). Langer et al. (*44*) presented data suggesting *de novo* methionine synthesis in an extract of *P. berghei*. However, the amount of product synthesized was low compared with a control lacking homocysteine, and more definitive data are needed before it can be assumed that these enzymes exist in *P. berghei*. In the experiments of Smith et al. referred to above (*43*), labelled methionine was obtained when the medium was

supplemented with 3-^{14}C serine or ^{35}S homocysteine, indicating the synthesis and utilization of N^5-methyltetrahydrofolate. It would be interesting to determine the relative importance of *de novo* methionine biosynthesis by plasmodia, since *P. knowlesi* requires methionine for optimum growth and incorporates exogenous methionine *in vitro* (see *7* for a review).

The synthesis and utilization of N^5-methyltetrahydrofolate by plasmodia would negate Platzer's hypothesis (*39*) that the " thymidylate cycle " was the only folate cofactor function in malaria. The possibility that plasmodia carry out folate-mediated functions other than the synthesis of thymidylate has been suggested by Coombs and by Gutteridge & Trigg. They present their own and other evidence to support the argument that DNA synthesis in plasmodia occurs mainly during the ring and trophozoite stages and little or not at all during the schizont stage (see *45* for a summary of this hypothesis). However, Conklin et al. (*46*) reported contradictory results—i.e., that the incorporation of orotic acid or adenosine into DNA occurred mainly during schizogony. These results bear directly on the proposed mode of action of antifolates and on the functions of folate cofactors. Many investigators have observed that inhibitory levels of sulfonamides or tetrahydrofolate dehydrogenase inhibitors cause the accumulation of abnormal-looking schizonts (*45*, *47-49*). If DNA synthesis occurred during schizogony, then the action of antifolates could be attributed to the inhibition of thymidylate synthesis by depletion of N^5,N^{10}-methylenetetrahydrofolate. However, Gutteridge & Trigg reported that pyrimethamine did not inhibit DNA synthesis (*28*). To explain these results they proposed that pyrimethamine does not gain entrance to the uninucleate parasite and thus does not inhibit the bulk of DNA synthesis; that the drug penetrates the schizont and inhibits the action of tetrahydrofolate dehydrogenase; and that the resultant depletion of tetrahydrofolate reduces the level of a folate cofactor that is involved in the process of schizogony (*50*). They suggested that methylation reactions might be involved, which would require the synthesis and utilization of N^5-methyltetrahydrofolate. The hypothesis can be tested experimentally by determining the permeability of different stages of plasmodia to pyrimethamine (and other tetrahydrofolate dehydrogenase inhibitors and sulfonamides, since they cause similar morphological effects) and by directly searching for the enzymes involved.

EFFECTS OF EXOGENOUS FOLATES IN MALARIA

Folic and folinic acids have been shown to aid malarial growth or survival and to reverse inhibition by sulfonamides and antifolates (*7*, *12*). However, most of the results reported are consistent with the hypothesis that cleavage products are utilized, not the intact molecules (see above). Trager (*51*) reported that the extracellular survival of *P. lophurae* on the fourth day of cultivation was aided by a high level of folinic acid. McCormack et al. (*35*) demonstrated stimulation of orotic acid incorporation into *P. knowlesi* DNA by pABA, folic acid, and folinic acid. These three compounds reversed the inhibition by sulfalene of orotic acid incorporation, but did not reverse the inhibition by pyrimethamine. The data from both studies could be explained by the presence in the media of pteridines and/or pABG, either as contaminants usually found in commercial samples of folic and folinic acids, or produced during the long incubations required for the studies. The reversal studies are a key to this phenomenon; if folinic acid were used intact, the inhibition of pyrimethamine would be reversed. Also, the reversal of sulfalene inhibition by folic and folinic acids should be noncompetitive—a prediction that can be experimentally tested. The competitive reversal by folic acid of sulfadiazine inhibition of *P. gallinaceum in vivo* led Rollo (*24*) to conclude that folic acid was broken down by the host to pABG.

The development of *P. falciparum in vivo* is not affected by folic or folinic acids. Hurly (*52*) was first to show that neither compound had an effect on the antimalarial action of pyrimethamine in man. More recently, similar results were observed with doses of folic and folinic acids that reversed the side-effects obtained with high-dose pyrimethamine therapy (*53*). Gail & Herms (*54*) treated *P. falciparum*-infected pregnant women with folic acid and observed a reticulocyte response without an effect on the parasite rate.

The reasons for the inability of malaria parasites to utilize exogenous, intact folates may vary with the species studied. Most microorganisms that synthesize folates *de novo* are impermeable to exogenous folates (*3*) and this is probably the case with plasmodia, although no direct evidence has been reported. It would be particularly important to study the uptake of N^5-methyltetrahydrofolate polyglutamates, since the erythrocytic parasites grow in a host cell rich in this cofactor (*26*). Reid & Friedkin (*25*) found that the total folate content of mouse blood infected

with *P. berghei* increased, but the most striking result was in the ratio of total folates to those with three or fewer glutamate residues. This ratio decreased as the parasitaemia increased. Trager (*55*) found large increases of folates in *P. lophurae*-infected duck blood, but did not determine the total folate content of the samples by treatment with an enzyme to break down the polyglutamates (conjugase). Thus it was not known of the total folates were increased in parasitized erythrocytes, or if a mere shift to the lower folate polyglutamates occurred. If these results can be taken to mean that higher polyglutamates of folates are not substrates for malarial enzymes, it is another reason why the host's folates are not utilized.

The lack of appropriate enzymes may explain why folic and folinic acids are not used intact by the parasites. Folic acid has not been found to be a substrate for malarial tetrahydrofolate dehydrogenase (*27*, *29*); folinic acid can be converted to N^{15}-formyltetrahydrofolate or N^5,N^{10}-methenyltetrahydrofolate by some tissues (*26*), but neither of these cofactors has been shown to be a substrate for malarial enzymes.

Lastly, the fact that the antifolates and the sulfonamides are so effective in inhibiting plasmodial growth argues against the utilization of the host's folate cofactors. The erythrocyte and the liver are rich sources of folate cofactors that could bypass the sites of action of these drugs. Yet they are effective in inhibiting the development of the parasite in both tissues. However, only studies on the uptake and utilization of exogenous folates can directly confirm or deny these speculations.

RÉSUMÉ

MÉTABOLISME DES FOLATES DANS LE PALUDISME

On sait depuis un certain temps maintenant que les parasites du paludisme sont inhibés par les sulfamides et par les antifoliques, qu'ils ont besoin d'acide amino-4 benzoïque pour leur croissance et qu'ils ne manifestent que des réponses partielles à l'égard des acides folique et folinique intacts. Les données biochimiques recueillies au cours des dix dernières années sur la synthèse des précurseurs de l'acide nucléique et sur les enzymes des folates dans le paludisme viennent à l'appui de l'hypothèse selon laquelle les parasites du paludisme ressemblent aux micro-organismes qui synthétisent *de novo* les cofacteurs des folates. Les sulfamides agissent par inhibition de la dihydropteroate-synthétase (EC 2.5.1.15) du plasmodium. La pyriméthamine et beaucoup d'autres antifoliques se lient à la tétrahydrofolate déshydrogénase (EC 1.5.1.3) du parasite plus étroitement qu'à l'enzyme de l'hôte. Toutefois, les conséquences métaboliques de l'épuisement des cofacteurs des folates à la suite de l'inhibition due aux médicaments ne sont pas encore connues. Les études devront porter aussi sur d'autres points, tels que: l'origine de la fraction ptéridine des folates, l'addition de glutamate(s) dans la biosynthèse des cofacteurs des folates; le mécanisme par lequel les folates intacts, exogènes, affectent la croissance des parasites du paludisme; et la mise en évidence des enzymes et des réactions qui font intervenir le N^5-méthyltétrahydrofolate.

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